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Original paper

Dialysis of flavour compounds: yields of extraction on model solution

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Dialyse von Aromaverbindungen. Ausbeute an Extrakt einer Modellösung

Zusammenfassung. Es wurde die Extraktion von 58 Aromasubstanzen durch Dialyse an einer Nafion-Membran unter Verwendung einer Modellösung untersucht. Die Reproduzierbarkeit und die Ausbeute der Methode wurden ermittelt. Der Variationskoeffizient der Extraktionsausbeute war kleiner als 15% für 48 der getesteten 58 Substanzen. Die Ausbeute verringert sich bei steigender Anzahl von Kohlenstoffatomen, und fällt auf 0 bei Substanzen mit mehr als 10 Kohlenstoffatomen. Aromasubstanzen mit ringförmiger Struktur zeigen eine bessere Diffusionsrate als andere Substanzen mit derselben Anzahl von Kohlenstoffatomen. Die Extraktionsausbeute scheint hauptsächlich mit der Hydrophobizität der Substanzen korreliert zu sein. Die Anwendbarkeit der Methode für die quantitative Analyse wurde untersucht. Die Extraktionsrate kann als unabhängig von der Substanzmenge betrachtet werden. Die Trennung von Aromasubstanzen und Lipiden mit dieser Methode wurde untersucht. Eine verbesserte Extraktionsausbeute wurde für 8 Substanzen beobachtet, für alle anderen Substanzen wurden keine signifikanten Unterschiede beobachtet. Das Öl diffundiert nicht durch die Dialysemembran.

Abstract. The extraction of 58 flavour compounds by Nafion membrane dialysis was studied using a model solution. Reproducibility and yield of the method were investigated. The coefficient of variation of the extraction yield was less than 15% for 48 of the 58 compounds tested. When the number of carbon atoms increased, the extraction yield fell to zero for compounds with more than ten carbon atoms. Flavour compounds with a cyclic structure showed a better diffusion rate through the membrane than other compounds with the same number of carbon atoms. The extraction yield seems to be correlated mainly to the hydrophobicity of the molecule. The use of the method for quantitative analysis was studied. Extraction rate can be considered as constant regardless of the quantity of compounds. The separation of flavour compounds from lipids was studied by this method. A better extraction yield with lipids was observed for eight compounds, for all other molecules no significant difference could be observed. The oil does not diffuse through the dialysis membrane.

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Introduction

The qualitative and quantitative analysis of flavour in fatty foods involves the separation of volatile compounds from the non-volatile fraction. Numerous techniques have been proposed, most of them taking advantage of the high volatility of the flavour compounds as compared to other components [1–3]. Two types of extraction can be considered:

1. High temperature techniques such as steam distillation of the Likens and Nickerson method [4]. These are subject to the formation of artifacts due to hydrolysis, oxidation and other reactions catalysed by heating.

2. Low temperature techniques such as direct two phase extraction methods (which are limited to samples with light fat content) or headspace techniques (the detection of flavour compounds released depends on their vapour pressure and is limited to highly volatile compounds). The high vacuum distillation methods [5] require a very specific material, are time-consuming and are unrepeatable, but, at the moment, they give the best results in terms of flavour profiles. All of these methods have some disadvantages, especially poor reproducibility. It would be interesting to improve soft undeveloped separation techniques.

Dialysis is a low temperature separation technique easy to use and based on molecular size differences. Flavour compounds are usually smaller than lipids and proteins. Because small molecules are able to diffuse through a specific membrane, it is possible to isolate flavour compounds from lipids by this technique. Benkler and Rei-

neccius [6, 7], Chum [8], Vandeweghe and Reineccius [9], used Nafion membranes (Du Pont De Nemours and Co, Fayetteville, N.C., USA) for the isolation of volatile compounds from fat. All the flavour compounds studied and only a small amount of oil diffused through the membrane. The solvent system used was an acetone/pentane mixture. This method had several problems such as a binding of basic compounds onto the membrane and some artifact formations. The use of 1% water in diethyl ether was found to inactivate acidic sites on the membrane and thus eliminate adsorption and artifact formation [10].

Until today, only a few reports on the separation of flavour compounds by dialysis have been published. In this paper we have investigated the yield and the repeatability of a dialysis extraction technique. A model solution of 58 flavour compounds in a fat matrix have been studied and the limits of the method discussed.

Materials and methods

Flavour compounds. Compounds were chosen in the different chemical classes frequently found in cheese flavours: acids, alcohols, aldehydes, esters, lactones, methyl ketones and were purchased at the highest grade of purity found on the chemical market.

Solvent system. All solutions were prepared with 1% ultrapure water (Milli-Q, Millipore, Bedford, Mass., USA) in bidistilled diethyl ether (S.D.S., Peypin, France) [10].

Membrane. Perfluorosulphonic acid membrane Nafion 117 (Du Pont De Nemours) was used. Prior to use, the membrane was cut into circles of 10 cm diameter and expanded by soaking in boiling distilled water for 40 min.

Dialysis unit. A schematic diagram of the dialysis unit is shown in Fig. 1. The two half cells (A and B) are filled with a feed tube. The feed parts (F) were closed by teflon stoppers. Nafion membrane (C) was placed between the two half cells and a magnetic stirrer (E) was placed in each partition. A locking mechanism (D) joined hermetically the two half cells. Each partition of the dialysis unit had a volume of 320 ml and the exchange surface was 30.2 cm^2.

Dialysis technique. Six hundred millilitres of 1% water diethyl ether per dialysis unit were prepared. This solution (300 ml) was used as extracting solvent in the first division (B) of the dialysis apparatus. Each flavour compound (2 mg) was dissolved in the other 300 ml. This solution was poured into the second partition (A). The dialysis unit was maintained for 72 h at 25° C under shaking. The two partitions were not hermetically closed, to prevent a change in pressure which would damage the membrane.

Analysis of flavour compounds. An internal standard (0.3 mg) was added to each partition of the dialysis unit. Internal standards used were the pentanoic acid pentyl ester (Fluka, Buchs, Switzerland) for neutral compounds and the 3-methyl-2-butenoic acid (Aldrich, Strasbourg, France) for acidic compounds. The solvent from each partition of the dialysis unit was dried with anhydrous sodium sulphate (Prolabo, Paris, France) and filtered with glassfibre (Prolabo). The filtrate was carefully concentrated down to 2 ml with a Kuderna-Danish column and down to 0.5 ml with a Dufton column at 45° C.

When a mixture of neutral and acidic compounds was dialysed, these were separated before analysis. Both of the two internal standards (0.3 mg), 100 ml of 300 g L^{-1} solution of sodium chloride (Prolabo) and 100 ml of a sodium hydroxide (Prolabo) solution, 0.01, were added to each partition of the dialysis unit. After

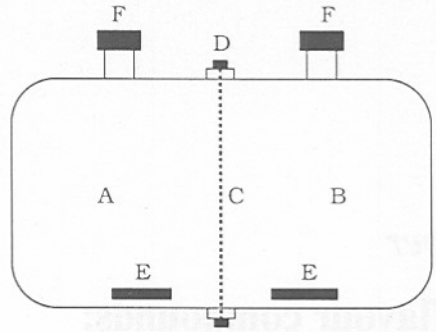

Fig. 1. Dialysis unit: *A, B,* two half cells; *C,* Nafion membrane; *D,* locking mechanism; *E,* magnetic stirrer; *F,* feed tube entrance closed by a Teflon stopper

10 min of shaking, the organic phase, containing the neutral compounds, and the aqueous phase, containing the acid salts, were separated in a separating funnel. The acidic compounds were extracted three times in a separating funnel with ethyl ether (1 × 60 ml and 2 × 40 ml) from the aqueous phase after regeneration from their salts at pH 1 with 1 mol hydrochloric acid (Prolabo). Both neutral and acidic solutions were treated as described previously, before analysis by gas chromatography (GC).

GC conditions. A DI 700 chromatograph (Perkin Elmer, Saint-Quentin-en-Yveline, France) equipped with a fused silica capillary column DB 1701, 30 m × 0.32 mm (i.d.) with a film thickness of 1 µm (J and W Scientific, Folsom, Calif., USA) was used for neutral compounds. A Girdel 300 chromatograph (Perkin Elmer) equipped with a fused silica capillary column DB-FFAP, 30 m × 0.32 mm (i.d.) and film thickness of 0.25 µm (J and W Scientific) was used for acidic compounds. In both cases the H_2 carrier gas velocity was 50 cm s^{-1}. The oven temperature was programmed from 30° C to 240° C at 2° C mn^{-1}. The flame ionisation detector and split-splitless injector temperatures were maintained at 250° C. GC data analysis was done using the personal-computer-based, precision multichannel chromatography workstation ("Coconut", Mielle and Almanza, INRA, Dijon, France).

Results and discussion

Measurement of reproductibility and yields of the dialysis

In a first step we studied the dialysis extraction of compounds belonging to different chemical families. Seven dialyses were done corresponding, respectively, to different families of molecules. Each dialysis was repeated three times. The coefficient of variation (CV) of the extraction yield was less than 15% for 48 compounds out of 58 compounds dialysed (Table 1). Methyl heptanoate, methyl octanoate, methyl nonanoate, methyl decanoate, δ- and γ-decalactone and δ-dodecalactone showed a CV of over 20%, probably due to their molecular size (more than eight carbons atoms) limiting their diffusion through the membrane. These results show the good repeatability of this method as compared to other flavour extraction methods. Gallois and Langlois [11] showed a CV higher than 20% for most flavour compounds that they isolated from French cheese using the Forss and Holloway distillation method [5]. At equilibrium, the maximum theoretical percentage of compounds on both

Table 1. Balance sheet after dialysis of flavour compounds

Number of dialysis	Compounds	LogP	Partition A % (C.V.)	Partition B % (C.V.)	Total (%)
	Acids				
1	Acetic acid	−0.3	29 (5.2)	45 (4.0)	74
1	Propanoic acid	0.2	40 (2.2)	54 (4.3)	94
1	Isobutyric acid	0.9	63 (4.9)	38 (4.4)	101
1	Butyric acid	0.8	70 (2.6)	43 (4.1)	113
1	Pentanoic acid	1.3	80 (2.6)	19 (3.1)	99
1	Hexanoic acid	1.8	92 (2.8)	5 (7.0)	97
1	Heptanoic acid	2.3	105 (2.2)	1 (12.8)	106
1	Octanoic acid	2.9	100 (3.5)	1 (14.1)	101
1	Nonanoic acid	3.4	99 (1.7)	1 (14.1)	100
1	Decanoic acid	3.9	99 (4.3)	1 (14.2)	100
1	Dodecanoic acid	5.0	82 (3.5)	1 (12.4)	83
	Alcohols				
2	1-Butanol	0.8	44 (5.8)	41 (6.4)	85
2	1-Pentanol	1.4	53 (3.7)	31 (10.8)	84
2	1-Hexanol	1.9	78 (5.3)	25 (2.2)	103
2	1-Heptanol	2.4	90 (1.1)	12 (6.3)	102
2	1-Octanol	3.0	91 (0.8)	5 (11.2)	96
2	1-Nonanol	3.5	95 (3.1)	0	95
2	1-Decanol	4.0	106 (2.5)	0	106
2	1-Undecanol	4.5	99 (1.4)	0	99
2	3-Methyl-1-butanol	1.3	49 (6.4)	54 (2.3)	103
2	1-Octen-3-ol	2.4	74 (4.9)	23 (3.8)	97
2	2-Phenylethanol	1.5	59 (5.3)	41 (6.6)	100
	Aldehydes				
3	Pentanal	1.9	71 (7.8)	9 (16.5)	80
3	Hexanal	2.4	94 (7.8)	11 (8.6)	105
3	Heptanal	2.9	74 (3.3)	15 (8.4)	89
3	Octanal	3.5	89 (3.7)	19 (15.1)	108
3	Nonanal	4.0	88 (4.0)	1 (11.5)	89
3	Decanal	4.6	87 (3.8)	1 (13.4)	88
3	Benzaldehyde	1.5	59 (5.5)	37 (3.5)	96
	Esters				
4	Ethyl propanoate	1.2	68 (14.2)	32 (7.1)	100
4	Ethyl butanoate	1.7	80 (8.7)	18 (13.5)	98
4	Ethyl pentanoate	2.2	79 (4.4)	7 (6.2)	86
4	Ethyl hexanoate	2.8	76 (1.8)	6 (8.8)	82
4	Ethyl heptanoate	3.3	91 (1.3)	4 (10.0)	95
4	Ethyl octanoate	3.8	92 (0.5)	2 (12.3)	94
4	Ethyl nonanoate	4.3	97 (1.5)	2 (12.2)	99
4	Ethyl decanoate	4.9	97 (1.3)	1 (11.9)	98
5	Methyl butanoate	1.2	73 (2.9)	36 (1.4)	109
5	Methyl hexanoate	2.2	87 (2.0)	19 (13.7)	106
5	Methyl heptanoate	2.8	90 (1.7)	12 (18.5)	102
5	Methyl octanoate	3.3	93 (0.8)	9 (22.6)	102
5	Methyl nonanoate	3.8	96 (2.5)	6 (27.2)	102
5	Methyl decanoate	4.3	95 (2.3)	4 (29.1)	99
5	2-Phenylethylacetate	2.4	89 (5.0)	6 (3.7)	95
	Lactones				
6	Gamma-butyrolactone	0.3	57 (5.5)	51 (1.3)	108
6	Gamma-decalactone	3.4	74 (7.3)	24 (28.5)	98
6	Delta-decalactone	3.4	70 (13.5)	21 (55.2)	91
6	Delta-dodecalactone	4.4	93 (9.8)	1 (70.7)	94
	Methylketones				
7	2-Pentanone	0.8	63 (2.9)	52 (7.2)	115
7	2-Hexanone	1.3	75 (0.4)	38 (8.4)	113
7	2-Heptanone	1.8	84 (4.4)	20 (3.1)	104
7	2-Octanone	2.4	99 (0.5)	12 (12.6)	111
7	2-Nonanone	2.9	91 (2.3)	6 (13.5)	97
7	2-Decanone	3.4	81 (0.1)	3 (16.4)	84
7	2-Undecanone	3.9	85 (0.2)	2 (18.0)	87
7	3-Methyl-2-pentanone	1.2	51 (8.0)	52 (4.9)	103
7	Acetophenone	0.9	72 (4.9)	41 (1.9)	113
6	Styrene	2.7	91 (4.2)	4 (7.2)	95

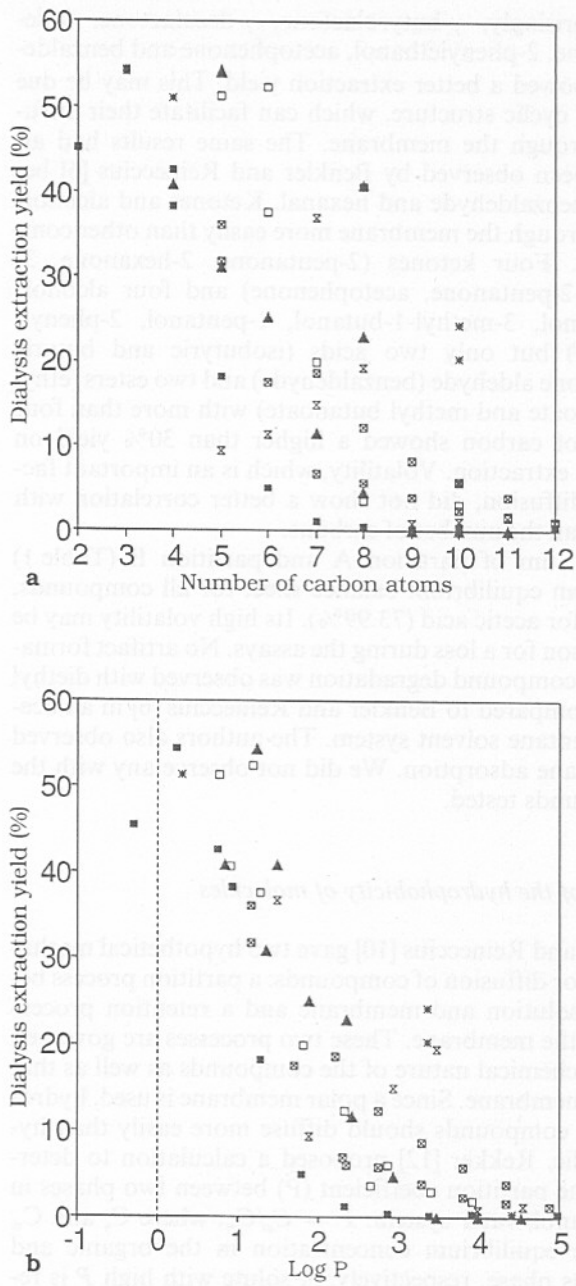

Fig. 2. Influence of the carbon atom number (**a**) and the logarithm of the hydrophobic fragmental constant (log P) (**b**) on the flavour compounds dialysis efficiency: ■ acids, ▲ alcohols, x aldehydes, ⊠esters, ⁎ lactones, □ ketones

sides of the membrane is 50%. As expected, this theoretical value was observed for the smaller molecules (two to five carbon atoms) such as acetic acid, propanoic acid, 2-pentanone, γ-butyrolactone (Table 1). When the number of carbon atoms increased, the yield of dialysis extraction fell to zero for the biggest compounds such as nonanoic acid, decanoic acid, dodecanoic acid, 1-nonanol, 1-decanol, 1-undecanol, δ-dodecalactone (Fig. 2 a). The correlation coefficient between the yield of dialysis and the number of carbons, with a value of 0.56 is significant with 56 degrees of freedom (df).

Surprisingly, γ-butyrolactone, γ-decalactone, δ-decalactone, 2-phenylethanol, acetophenone and benzaldehyde showed a better extraction yield. This may be due to their cyclic structure, which can facilitate their diffusion through the membrane. The same results had already been observed by Benkler and Reineccius [6] between benzaldehyde and hexanal. Ketones and alcohols went through the membrane more easily than other compounds. Four ketones (2-pentanone, 2-hexanone, 3-methyl-2-pentanone, acetophenone) and four alcohols (1-butanol, 3-methyl-1-butanol, 1-pentanol, 2-phenylethanol) but only two acids (isobutyric and butyric acids), one aldehyde (benzaldehyde) and two esters (ethyl propanoate and methyl butanoate) with more than four atoms of carbon showed a higher than 30% yield on dialysis extraction. Volatility, which is an important factor in diffusion, did not show a better correlation with yield than the number of carbons.

The sum of partition A and partition B (Table 1) shows an equilibrium balance sheet for all compounds, except for acetic acid (73.99%). Its high volatility may be the reason for a loss during the assays. No artifact formation or compound degradation was observed with diethyl ether compared to Benkler and Reineccius [6] in an acetone/pentane solvent system. The authors also observed membrane adsorption. We did not observe any with the compounds tested.

Effect of the hydrophobicity of molecules

Chang and Reineccius [10] gave two hypothetical mechanisms for diffusion of compounds: a partition process between solution and membrane and a retention process within the membrane. These two processes are governed by the chemical nature of the compounds as well as that of the membrane. Since a polar membrane is used, hydrophobic compounds should diffuse more easily than hydrophilic. Rekker [12] proposed a calculation to determine the partition coefficient (P) between two phases in an octanol/water system: $P = C_s/C_w$, where C_s and C_w are the equilibrium concentration in the organic and aqueous phase, respectively. A solute with high P is regarded as lipophilic and a solute with low P as hydrophilic. As the P scale covers a range of more than 10^{10}, logarithmic P values are usually preferred. $\log P = \log C_s - \log C_w = \Sigma a_n f_n$, where f represents the hydrophobic fragmental constant, the lipophilicity contribution of a constituent part of a structure to the total lipophilicity, and a is a numerical factor indicating the incidence of a given fragment in the structure.

We considered the solvent/membrane system as a two-phase system and we tried to link the dialysis efficiency with the $\log P$ of the dialysed flavour compounds (Fig. 2b). This data analysis improved the correlation coefficient to 0.71 (56 df) and permitted consideration of the general behaviour of the different chemical families of compounds. The yield seems to correlate mainly to its $\log P$. The diffusivity of a given compound increased with increased polarity and decreased molecular size. As a consequence, the value of $\log P$ permitted a rough estimation of the molecular behaviour. All the compounds studied with a $\log P$ lower than 1.6 showed a higher than 30% extraction yield. The extraction yield was close to zero for compounds with a $\log P$ value above four. However lactones seemed to have a different behaviour as shown in Fig. 2b. The extraction yield decreased more slowly than the other chemical families.

Use of the method for quantitative analysis

Solutions containing a mixture of ten neutral compounds and eleven acid compounds were dialysed. Four quantities (0.5 mg, 1.0 mg, 1.5 mg and 2.0 mg) were tested and each dialysis was repeated three times. The results are presented in Table 2. A variance analysis testing the CV of yields as a function of the quantity dialysed did not show differences in accuracy between 0.5 mg, 1.0 mg, 1.5 mg and 2.0 mg. As a consequence, extraction yield after a 3-day dialysis can be considered as constant if the quantity dialysed is between 0.5 and 2.0 mg. Benkler and Reineccius [6] reported that a significant decrease in diffusion in the presence of 2-methoxypyrazine may be due to obstruction of the pores of the membrane. Those authors observed the disappearance of the 2-methoxypyrazine during the dialysis, attributed to either 2-methoxypyrazine adsorption or reaction with the membrane. Because we mixed acids and neutral compounds, it was difficult to quantify all volatiles in the same way. Con-

Table 2. Dialysis extraction yield according to the quantity of flavor compounds dialyses

Compounds	Quantity dialysed (mg)							
	0.5		1.0		1.5		2.0	
	(%)	(CV)	(%)	(CV)	(%)	(CV)	(%)	(CV)
Acid compounds								
Acetic acid	96	(14.8)					106	(3.1)
Propanoic acid	74	(7.4)	87	(6.0)	89	(1.8)	61	(6.2)
Isobutyric acid	60	(4.2)	62	(3.1)	65	(0.5)	44	(5.1)
Butyric acid	64	(2.6)	61	(10.5)	68	(1.0)	70	(2.5)
Pentanoic acid	37	(3.0)	38	(2.5)	38	(2.1)	40	(4.1)
Hexanoic acid	19	(3.6)	18	(2.9)	17	(0.5)	20	(3.1)
Heptanoic acid	9	(10.4)	8	(4.8)	8	(6.0)	9	(5.7)
Octanoic acid	5	(9.2)	4	(5.5)	4	(6.4)	6	(4.3)
Nonanoic acid	3	(19.3)	2	(11.2)	2	(6.6)	2	(2.8)
Decanoic acid	2	(20.7)	1	(9.5)	1	(2.2)	2	(11.3)
Dodecanoic acid	1	(71.6)	1	(25.0)	0		1	(33.9)
Tridecanoic acid	2	(141.4)	1	(141.5)	0		2	(71.8)
Neutral compounds								
3-Methyl-1-butanol	58	(4.8)	65	(1.6)	62	(8.9)	63	(4.5)
Hexanal	45	(6.2)	46	(3.1)	48	(2.8)	39	(5.0)
2-Heptanone	37	(4.6)	37	(3)	53	(34.7)	26	(60.1)
Benzaldehyde	43	(2.6)	42	(2.9)	45	(1.7)	42	(3.0)
1-Octen-3-ol	30	(2.8)	29	(3.1)	30	(2.2)	21	(5.8)
2-Nonanone	9	(2.0)	9	(4.2)	9	(3.5)	9	(2.8)
Methyl nonanoate	2	(6.5)	2	(5.4)	1	(71.0)	2	(3.3)
Ethyl decanoate	1	(72.7)	1	(17.9)	1	(5.1)	1	(6.8)
γ-Decalactone	22	(2.6)	12	(3.8)	20	(5.2)	20	(4.5)
Methyl-hexadecanoate	1	(101.2)	1	(42.2)	0		0	

sequently we do not know if the change in extraction yields (with the results presented above) should be attributed to interactions between compounds or to the methodology. The mean yield obtained with the first method is significantly higher than that obtained by the second method. It could be due to a loss during the partition between acid compounds and neutral compounds. We did not observe adsorption to or reaction with the membrane. Obstruction of the pores of the membrane does not seem to explain this result.

Influence of lipids

As this method could be especially interesting for use with food-containing lipids, we looked for the effect of lipids in model solutions. Two series of three dialyses on the same 21 flavour compounds with glycerol-trioleate (20 g; Prolabo) were done. A variance analysis using Scheffe statistical test (S.A.S., S.A.S. Institute, Cary, N.C., USA), showed a better extraction yield when lipids were added for eight compounds (Table 3). For all other molecules no significant difference could be observed. To explain this behaviour, two hypotheses can be formulated. First, the formation of a limit layer at the surface of the membrane improves mass transfer of lipophilic compounds. Second, an improvement in the transfer from partition A to partition B restores the concentration equilibria. As the compounds for which the yields were improved were small molecules with a small $\log P$, the second hypothesis is preferred. The CV were not affected by lipids in the solution. The GC analysis of the residue after ethyl ether evaporation from partition B revealed that the glycerol-trioleate did not diffuse through the membrane. Benkler and Reineccius [6] have already shown that less than 0.12% of the corn oil added diffused through the membrane after an 8-day dialysis.

Table 3. Significant effect of lipids on dialysis extraction yield

Compounds	Increase in extraction yield (%)
Hexanal	26.5
Benzaldehyde	5.3
Acetic acid	37.6
Propanoic acid	49.1
Isobutyric acid	43.8
Butyric acid	13.5
Pentanoic acid	25.0
Hexanoic acid	20.5

The above results were very encouraging for the use of this technique in the extraction of food flavours especially in foods containing lipids. However, it would be interesting to improve transfer yield and transfer kinetics to be able to quantify flavours in serial measurements. As in all membrane systems, an improvement may be obtained by increasing the surface/volume ratio, the concentration gradient and the temperature. All these factors have not yet been optimized. Chang and Reineccius [10] used a countercurrent continuous dialysis system to increase the concentration gradient and dialysis tubing to increase the surface/volume ratio. The dialysis rate was noticeably increased compared to the batch method, and the dialysis time reduced. However, the method could not allow work with a small concentrated sample due to the dead volume of the apparatus.

The general behaviour of the molecules presented here shows that the primary mechanism affecting transfer in dialysis through Nafion membrane is the hydrophobicity of the molecules. With this knowledge such an optimization should be easier, and permit the results obtained with a chemical family to be generalized to all the families studied.

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